Acute and sustained effects of methylphenidate on cognition and presynaptic dopamine metabolism: An [18F]FDOPA PET study

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Abstract: Methylphenidate (MPH) inhibits the reuptake of dopamine and noradrenaline. PET studies with MPH challenge show increased competition at postsynaptic D2/3-receptors, thus indirectly revealing presynaptic dopamine release. We used [(18)F]fluorodopamine ([(18)F]FDOPA)-PET in conjunction with the inlet-outlet model (IOM) of Kumakura et al. (2007) to investigate acute and long-term changes in dopamine synthesis capacity and turnover in nigrostriatal fibers of healthy subjects with MPH challenge. Twenty healthy human females underwent two dynamic [(18)F]FDOPA PET scans (124 min; slow bolus-injection; arterial blood sampling), with one scan in untreated baseline condition and the other after MPH administration (0.5 mg/kg, p.o.), in randomized order. Subjects underwent cognitive testing at each PET session. Time activity curves were obtained for ventral putamen and caudate and were analyzed according to the IOM to obtain the regional net-uptake of [(18)F]FDOPA (K; dopamine synthesis capacity) as well as the [(18)F]fluorodopamine washout rate (kloss, index of dopamine turnover). MPH substantially decreased kloss in putamen (-22%; $p = 0.003$). In the reversed treatment order group (MPH/no drug), K was increased by 18% at no drug follow-up. The magnitude of K at the no drug baseline correlated with cognitive parameters. Furthermore, individual kloss changes correlated with altered cognitive performance under MPH. [(18)F]FDOPA PET in combination with the IOM detects an MPH-evoked decrease in striatal dopamine turnover, in accordance with the known acute pharmacodynamics of MPH. Furthermore, the scan-ordering effect on K suggested that a single MPH challenge persistently increased striatal dopamine synthesis capacity. Attenuation of dopamine turnover by MPH is linked to enhanced cognitive performance in healthy females.

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Behavioral/Cognitive

Acute and Sustained Effects of Methylphenidate on Cognition and Presynaptic Dopamine Metabolism: An [^{18}F]FDOPA PET Study

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Methylphenidate (MPH) inhibits the reuptake of dopamine and noradrenaline. PET studies with MPH challenge show increased competition at postsynaptic $D_{2/3}$-receptors, thus indirectly revealing presynaptic dopamine release. We used [^{18}F]fluorodopamine ([^{18}F]FDOPA)-PET in conjunction with the inlet–outlet model (IOM) of Kumakura et al. (2007) to investigate acute and long-term changes in dopamine synthesis capacity and turnover in nigrostriatal fibers of healthy subjects with MPH challenge. Twenty healthy human females underwent two dynamic [^{18}F]FDOPA PET scans (124 min; slow bolus-injection; arterial blood sampling), with one scan in untreated baseline condition and the other after MPH administration (0.5 mg/kg, p.o.), in randomized order. Subjects underwent cognitive testing at each PET session. Time activity curves were obtained for ventral putamen and caudate and were analyzed according to the IOM to obtain the regional net-uptake of [^{18}F]FDOPA (K; dopamine synthesis capacity) as well as the [^{18}F]fluorodopamine washout rate (k_{loss}, index of dopamine turnover). MPH substantially decreased k_{loss} in putamen (-22%; $p = 0.003$). In the reversed treatment order group (MPH/no drug), K was increased by 18% at no drug follow-up. The magnitude of K at the no drug baseline correlated with cognitive parameters. Furthermore, individual k_{loss} changes correlated with altered cognitive performance under MPH. [^{18}F]FDOPA PET in combination with the IOM detects an MPH-evoked decrease in striatal dopamine turnover, in accordance with the known acute pharmacodynamics of MPH. Furthermore, the scan-ordering effect on K suggested that a single MPH challenge persistently increased striatal dopamine synthesis capacity. Attenuation of dopamine turnover by MPH is linked to enhanced cognitive performance in healthy females.

Key words: **[^{18}F]FDOPA PET; cognition; dopamine turnover; long-term effects; methylphenidate; stimulants**

Introduction

Methylphenidate (MPH) facilitates dopaminergic transmission by inhibiting the dopamine reuptake transporter (DAT). In clinical practice, MPH is treatment of choice in attention deficit/hyperactivity disorder (ADHD). Because of its straightforward pharmacodynamic mechanism, MPH has also been used as a challenge in PET investigations inducing increased competition between $D_{2/3}$-receptor ligands and endogenous dopamine (Volkow et al., 1994; Rosa-Neto et al., 2005). This MPH-induced reduction of the ligand binding potential, however, is a surrogate parameter of the changes in dopamine concentrations and is confounded by some biological processes, such as receptor internalization (Skinbjerg et al., 2010). The present approach using [^{18}F]fluorodopamine ([^{18}F]FDOPA) does not target the estimation of dopamine release but mimics the presynaptic synthesis pathway of dopamine. Given that the primary application of MPH (ADHD) is claimed to be characterized by presynaptic dis-

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turbances (e.g., Ernst et al., 1998; Volkow et al., 2007; Ludolph et al., 2008), the evaluation of MPH effects by a predominantly presynaptic tracer appears to be reasonable.

Until now, [18F]FDOPA PET was not used for the evaluation of MPH effects; it traces the dopamine synthesis capacity in brain, which appears to be less vulnerable for environmental and pharmacological influences (Gründer et al., 2003; Vernaleken et al., 2013). However, most quantitative FDOPA PET studies assume irreversible trapping of [18F]FDOPA, whereas the inlet–outlet model (IOM) of Kumakura et al. (2007) yields the net clearance of [18F]FDOPA to brain (K; ml · g^{-1}min^{-1}), accommodating the delayed washout of decarboxylated and deaminated metabolites, which is explicitly defined by the rate constant k_{loss} (min). Thus, the [18F]FDOPA IOM can reveal acute changes in the dopamine dynamics.

Using this model, the present investigation intends to monitor the acute and delayed effects of MPH on dopamine turnover (k_{loss}) and dopamine synthesis capacity (K) as well as respective correlations with cognitive changes. Because DAT inhibition will reduce the rate of dopamine reuptake into the cytosolic compartment where it would be exposed to monoamine oxidase (Nielsen et al., 1983; Zetterström et al., 1988), we hypothesize that the magnitude of k_{loss} will be reduced. We furthermore expect that changes in k_{loss} correlate with enhancement in cognition under MPH treatment.

Therefore, two [18F]FDOPA PET scans (A: untreated control condition; B: scan after MPH challenge) were conducted in healthy subjects. Because long-term effects of psychostimulants on dopamine transmission and sensitization are known (Calipari et al., 2014), this study was conducted with two treatment order groups (no drug/MPH and MPH/no drug) to obtain possible regulatory effects of a single MPH dose on presynaptic dopamine metabolism.

Materials and Methods

Subjects. Twenty healthy, nonsmoking female subjects 21–28 years of age (mean ± SD, 24.0 ± 1.9 years) were included in the study, which had been approved by the Research and Ethics Committees of the University Hospital of RWTH Aachen University. All subjects provided written informed consent. Exclusion criteria for the volunteers included current neurological, psychiatric, or systemic disease, pregnancy (13 of 20 subjects were using oral contraceptives), and current use of drugs affecting the CNS. All 20 subjects are a small subgroup of a large neurogenetic study group of women ($n = 200$) not including molecular imaging techniques (Schabram et al., 2013). The cohort is well characterized in respect to demographics, and by intention highly homogeneous in age, gender, and education. Men were not included to reduce heterogeneity and to improve the statistical power for main effects. The 20 PET subjects were randomly distributed into two treatment groups (usual or reversed order of baseline/MPH PET scans). To optimize statistical power for the two main hypotheses, the groups were asymmetrically distributed, with 14 receiving first the unmedicated control scan, followed by the MPH condition, and six being scanned in reversed order.

Neuropsychology. To investigate the prefrontal cognitive capacities of the participants in the two scanning conditions, we administered the Trail Making Test (TMT-A+B) of executive functioning (Reitan, 1955), Stroop test (Bäumler, 1985), d2-concentration test of attention (Brickenkamp, 2002), and degraded stimulus continuous performance task (dsCPT) for measuring attention, in which subjects need to react on either a degraded or contoured target stimulus (Schabram et al., 2013). Following upon Kathmann et al. (1996), dsCPT was analyzed according to the signal detection theory (SDT) (Peterson et al., 1954). The test parameters for hits, missed, false alarms, and correct rejections were entered into analysis using BayesSDT software package for MATLAB (Lee, 2008) to obtain the following primary outcome variables: discriminability (sensitivity index, d′) and decision-bias (β). This bias indicates either a more liberal (negative values) or a more conservative (positive values) trend toward accepting a stimulus as a target and is independent of the overall performance. The first part (d2 test and TMT) of neuropsychological testing was performed ~1 h before PET scan, whereas for technical reasons the dsCPT was performed after scan.

MPH challenge. Participants received MPH (Ritalin, Novartis Pharmaceuticals) at a dose of 0.5 mg/kg adjusted for body weight either at first ($n = 6$) or second PET scan ($n = 14$). Based upon pharmacokinetics of oral MPH, the drug was administered 2 h before tracer injection (Markowitz et al., 2003). Because of legal and institutional restrictions, it was not possible to include a placebo drug formulation in the study design. The medication condition was therefore not blinded.

PET scanning and plasma sampling procedure. Before each PET scan, a pregnancy test was performed. [18F]FDOPA scans were recorded with the Siemens ECAT HR+ whole-body PET, which has a field of view of 16.2 cm in 47 planes, an interplane spacing of 3.375 mm, and an axial resolution of 5.4 mm FWHM. Decarboxylation of [18F]FDOPA in peripheral tissues was inhibited by oral administration of carbidopa (Merck Sharp & Dome, 2 mg/kg body weight), of which two-thirds were given 1 h after and one-third before start of the emission recording. After a brief attenuation scan, a dynamic emission sequence lasting 124 min began upon intravenous injection of [18F]FDOPA at a dose of 226 ± 21 MBq (range, 166–263 MBq) as a slow bolus. Frame length increased progressively according to the following schedule: 3 × 20 s; 3 × 1 min, 3 × 2 min, 3 × 3 min, 15 × 5 min, 3 × 10 min interval (Gründer et al., 2003). Blood was automatically drawn from a radial arterial catheter (first 10 min), and the radioactivity concentration measured at 1 s intervals with an online γ-counter (Allogg ABSS V3) cross-calibrated to the tomograph. Subsequently, a series of 15 arterial blood samples are drawn manually, and their radioactivity concentrations measured using a well counter (PerkinElmer Wizzard2 gamma-counter). The fractions of untransformed [18F]FDOPA and its major plasma metabolite 3-*O*-methyl-[18F]-fluorodopa ([18F]OMFD) were measured by reverse-phase high performance liquid chromatography (Cumming et al., 1993) in plasma extracts prepared from arterial blood samples. The continuous plasma fractions of [18F]FDOPA and OMFD were calculated by interpolation of biexponential functions fitted to the measured fractions, and the two input functions calculated by multiplication with the total blood curve (Gillings et al., 2001).

PET data analysis. Emission images were reconstructed by filtered back projection with a 4 mm Hanning filter. The dynamic sequence was frame-wise corrected for head motion, using an interframe rigid-body transformation implemented in PMOD (Version 3.4, PMOD Technology). For spatial normalization, the summed images were first coregistered to the individual MR (1.5T MRT Scanner; Philips Gyroscan NT; Philips Medical Systems), and the MR-registered sequence was then normalized to the ICBM-452 template (Mazziotta et al., 2001) using PMOD (Brain Normalization II routine), and a 12 parameter rigid-body transformation. Decay-corrected time activity curves (TACs) were then calculated for a set of volume of interest templates, including cerebellum, and left and right ventral caudate nucleus and ventral putamen. For one participant with contraindications against MR tomography, PET images were registered to a normalized [18F]FDOPA-template.

[18F]FDOPA kinetics. Most brain [18F]FDOPA studies are quantified by linear graphical analysis relative to the arterial [18F]FDOPA input, or a reference tissue surrogate. With PET recordings of 45–60 min, graphical analysis yields an index of [18F]FDOPA utilization that assumes irreversible trapping, thus ignoring the rapid formation of deaminated [18F]FDOPA metabolites in living striatum, and their diffusion from brain (Cumming et al., 1993). Because this metabolic process entails useful information about the turnover of the neurotransmitter pool, we elected to use the reversible IOM (Kumakura et al., 2007) for kinetic analysis of the regional TACs. The IOM is based on principles similar to those of the reversible tracer model of Sossi et al. (2001), as also used by Cumming et al. (2001); both approaches yield outcome parameters for the net blood–brain clearance of [18F]FDOPA, a fractional rate constant for the diffusion from brain of deaminated [18F]FDOPA metabolites, and also a distribution volume, which reflects dopamine storage capacity. The approach of Sossi et al. (2001) necessarily entails PET acquisition times of 4 h, whereas the presents IOM applies for recordings of only 2 h

Table 1. Cognitive performance scores

	No drug	N	MPH condition	N	Change (Δ)	% Change	Z	p
IQ	110.4 ± 12.1	14						
TMT-A (s)	17.5 ± 4.5	19	16.4 ± 3.5	20	−1.17	−6.67	−1.187	0.235
TMT-B (s)	34.1 ± 9.2	19	33.5 ± 9.8	20	−0.62	−1.82	−0.523	0.601
Stroop interference	30.0 ± 8.1	20	29.2 ± 8.1	20	−0.74	−2.47	−0.709	0.478
Decision bias β	0.64 ± 0.90	19	0.34 ± 0.088	20	−0.3	−53.13	−1.933	0.053
Sensitivity d′	2.00 ± 0.90	19	2.23 ± 0.93	20	0.23	11.50	−1.023	0.306
dsCPT hits	6.74 ± 2.02	19	7.20 ± 1.79	20	0.46	6.82	−0.742	0.458
d2 concentration performance	246.0 ± 30.9	19	260.8 ± 28.1	20	14.8	6.02	−2.865	0.004*

*$p < 0.05$.

Table 2. Effect of MPH challenge on [18F]FDOPA PET kinetic parameters

	CTR, no drug condition			MPH, methylphenidate condition			%Change: MPH-CTR	Z	p
	Mean	SD	N	Mean	SD	N			
k_{loss}									
CN right	0.0036	0.0015	19	0.0028	0.0009	20	−22%	−1.972	0.048*
CN left	0.0035	0.0015	20	0.0031	0.0011	20	−10%	−1.157	0.247
CN bilateral	0.0036	0.0014	19	0.0030	0.0009	20	−17%	−1.932	0.053
PUT right	0.0037	0.0011	19	0.0031	0.0011	20	−15%	−2.093	0.036*
PUT left	0.0037	0.0014	20	0.0029	0.0008	20	−22%	−2.949	0.003**
PUT bilateral	0.0038	0.0012	19	0.0030	0.0008	20	−20%	−2.656	0.007**
K									
CN right	0.0198	0.004	19	0.0196	0.003	20	−1%	−0.322	0.748
CN left	0.0201	0.005	20	0.0198	0.003	20	−1%	−0.299	0.765
CN bilateral	0.0200	0.005	19	0.0197	0.003	20	−2%	−0.040	0.968
PUT right	0.0226	0.005	19	0.0224	0.004	20	−1%	−0.080	0.936
PUT left	0.0224	0.005	20	0.0216	0.003	20	−4%	−0.672	0.502
PUT bilateral	0.0226	0.005	19	0.0220	0.003	20	−3%	−0.161	0.872
V_d									
CN right	7.05	3.37	19	7.73	2.36	20	10%	−1.288	0.198
CN left	7.41	3.25	20	7.41	3.25	20	0%	−0.560	0.575
CN bilateral	6.96	3.28	19	7.57	2.74	20	9%	−1.046	0.295
PUT right	6.94	2.78	19	8.19	2.85	20	18%	−1.569	0.117
PUT left	7.42	3.94	20	8.30	2.82	20	12%	−1.680	0.093
PUT bilateral	7.14	3.23	19	8.24	2.60	20	16%	−2.012	0.044*

CTR, No drug condition; CN, caudate nucleus; PUT, putamen; MPH, methylphenidate condition.

*$p < 0.05$; **$p < 0.01$.

because of a more accurate subtraction of brain radioactivity arising from the peripheral metabolite OMFD. The IOM entails a three-step approach in which a constrained one tissue compartment model is first used to calculate the TAC for plasma-derived [18F]OMFD in cerebellum, which contains negligible DOPA-decarboxylase activity. Here the permeability ratio (q) for the two substances arising from blood (OMFD/FDOPA) is a fixed parameter (Gjedde et al., 1991; Huang et al., 1991), set to a magnitude of 1.5, which is the mean of the limited number of explicit measurements (Cumming and Gjedde, 1998). Assuming, like all [18F]FDOPA models, homogeneous distribution of [18F]OMFD throughout brain, the curve calculated in cerebellum is then subtracted from the entire 4D PET recording, to isolate the brain contents of [18F]FDOPA, [18F]FDOPA, and its deaminated metabolites, which freely diffuse from brain. The first 20 min are excluded from the IOM analysis because of the need for an equilibrium for [18F]FDOPA in brain (Kumakura et al., 2007). Finally, the multilinear form of the IOM is applied to the "cleaned" brain TACs to calculate the steady-state parameters alluded to above: (1) the net blood–brain clearance of [18F]FDOPA (K, ml hg^{-1} min^{-1}), which is an index of dopamine synthesis capacity; (2) the washout rate for [18F]FDOPA together with its deaminated metabolites (k_{loss}; min^{-1}), which is comparable with the biochemical assays of dopamine turnover; and (3) the steady-state distribution volume of [18F]FDOPA together with its decarboxylated metabolites (V_d; ml/g), which is an index of dopamine storage capacity comparable with the effective distribution volume (EDV; ml · g^{-1}) defined by Sossi et al. (2001).

Statistical analyses. Wilcoxon's rank order tests for paired samples were conducted to investigate effects of MPH on cognition and PET parameters (K, k_{loss}, and V_d). The baseline versus MPH condition differences (percentage of the change) were calculated as ([no drug − MPH]/no drug × 100). Δ parameters were calculated for K, k_{loss}, and the cognitive measures (MPH condition subtracted by drug-free condition) indicating the percentage of change. To examine the order of treatment effect, an independent t test was conducted for Δ-K and Δ-k_{loss}. To justify a deeper investigation for treatment order effect, a prescreening threshold of $p < 0.2$ was applied for the decision to include parameters in the repeated-measures ANOVA (i.e., K and treatment order). Furthermore, Spearman correlations between baseline PET parameters and baseline neuropsychology scores, Δneuropsychology scores, Δ-K and Δ-k_{loss} were calculated. To correct for multiple testing, a Bonferroni correction at $\alpha = 0.05$, calculated by the Dubey/Armitage-Pamar α boundary (Sankoh et al., 1997) was used. This correction includes the correlation among the regions.

Results

All 20 subjects successfully completed the two PET scans. In two subjects, neuropsychological testing (d2 test, TMT-A+B) was lost because of technical problems. Mean IQ was 110.43 ± 12.13 (SD) (Table 1). The mean (SD) specific activity of fluorine-18 was 9.4 ± 1.9 MBq/μmol (range 5.7–13.6 MBq/μmol), indicating a total injected mass of ~4 pmol; there were no significant differences in specific activity between the two PET scans ($T = 1.327$, $p = 0.231$). The mean (SD) dose of MPH was 33.0 ± 6.4 mg (range 20–50 mg). There was no group differences in the stage of menstrual cycle at the scanning day, nor any main effect of cycle or oral contraceptive use on any [18F]FDOPA PET parameter at baseline.

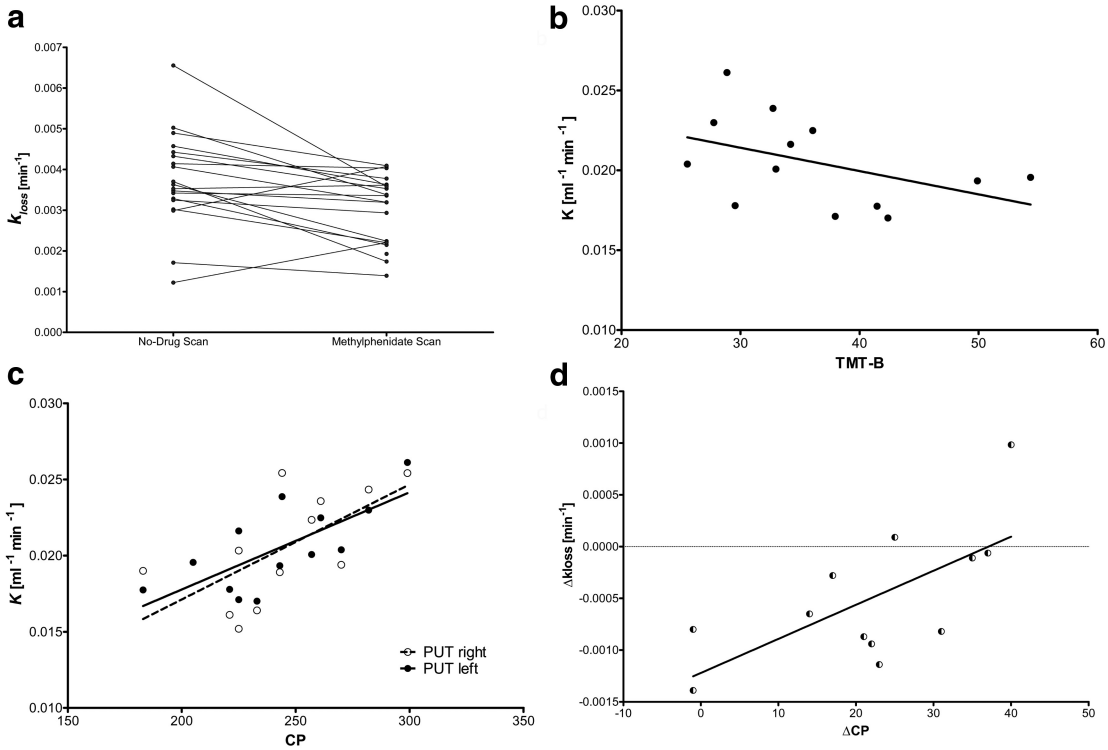

Figure 1. ***a***, Reductions in [18F]FDOPA k_{loss} in bilateral putamen after MPH challenge ($p = 0.007$, $n = 19$, complete sample). ***b***, Correlation between time at Trail Making Test B and no drug [18F]FDOPA *K* in left putamen ($p = 0.029$, $n = 14$, conventional order group). ***c***, Correlation between no drug [18F]FDOPA *K* and concentration performance parameter (CP) in right and left putamen (PUT) (right: $p = 0.039$, $n = 12$; left $p = 0.007$, $n = 13$, conventional order group). ***d***, Correlation between change in [18F]FDOPA k_{loss} and change in concentration performance under MPH ($p = 0.040$, $n = 12$, conventional order group).

Neuropsychological measurements

The results of the neuropsychological tests are displayed in Table 1. MPH increased concentration performance parameter (CP) in the d2 task (control condition: 245.95 ± 30.86, MPH: 260.75 ± 28.06 (mean ± SD); −6.0%, $Z = -2.87$, $p = 0.004$). However, there was no effect of MPH on TMT or dsCPT parameters.

PET parameters baseline

The mean (SD) magnitudes of [18F]FDOPA IOM parameters k_{loss}, *K*, and V_d in ventral caudate nucleus and in ventral putamen are reported in Table 2. The baseline k_{loss} was 0.0036 ± 0.0014 min^{-1} in the bilateral caudate nucleus and 0.0038 ± 0.0012 min^{-1} in the bilateral putamen. There were no significant side differences. Baseline *K* was 0.0200 ± 0.005 ml · g^{-1} · min^{-1} in the bilateral caudate nucleus and 0.0226 ± 0.005 ml · g^{-1} · min^{-1} in the bilateral putamen. Mean (SD) baseline V_d was 6.96 ± 3.28 ml · g^{-1} in the bilateral caudate nucleus and 7.14 ± 3.23 ml · g^{-1} in the bilateral putamen. In one participant, two ROIs (caudate right and putamen right) were excluded from consideration because of poor fitting outcome.

Effect of MPH on PET parameters

In the MPH challenge condition, mean (±SD) k_{loss} was 0.003 ± 0.0009 min^{-1} in bilateral caudate nucleus and 0.003 ± 0.0008 min^{-1} in bilateral putamen. Thus, MPH reduced k_{loss} by 22% in right caudate nucleus ($Z = -1.97$, $p = 0.048$, $n = 19$), 22% ($Z = -2.95$, $p = 0.003$, $n = 20$) in left putamen, and 15% ($Z = -2.09$, $p = 0.036$, $n = 19$) in right putamen (Table 2; Fig. 1*a*). Mean (SD) *K* was 0.0197 ± 0.003 ml · g^{-1} · min^{-1} for bilateral caudate nucleus and 0.0220 ± 0.003 ml · g^{-1} · min^{-1} for bilateral putamen; there were no significant effects of MPH on *K* ($n = 20$).

Table 3. ANOVA for [18F]FDOPA *K* in PUT and CN

	df	F	*p*
CN			
[18F]FDOPA *K*	1	0.45	0.511
Treatment order	1	3.098	0.095
K × TO	1	4.325	0.052
PUT			
[18F]FDOPA *K*	1	2.291	0.147
Treatment order	1	6.042	0.024*
K × TO	1	8.122	0.011*

PUT, Putamen; CN, caudate nucleus; TO, treatment order.
*$p < 0.05$.

Order of treatment effect

To test whether the order of the drug treatment had an effect on the PET parameters, we first performed an independent-sample *t* test; this showed that Δ*K* in bilateral caudate nucleus and bilateral putamen differed between groups (caudate: T = 1.90, $p = 0.075$ $n = 19$; putamen: $t = 2.90$, $p = 0.010$, $n = 19$). The Δ-k_{loss} parameter showed no general effect of treatment order, except in left putamen (T = 2.589, $p = 0.019$, $n = 20$). Because of these results, we performed repeated-measures ANOVAs, including *K* as a within-subjects variable and "order of treatment" as a between-subject variable. The tests revealed no main effect of *K* but a main effect of order of treatment and an interaction effect in bilateral putamen ($F = 8.426$, $p = 0.01$*; *K**order of treatment; see also Table 3). Bilateral caudate nucleus showed a trend in order of treatment effect. The *post hoc* analyses revealed that, in those subjects with reversed order of drug application (MPH scan first), baseline *K* estimates in bilateral putamen were 24% higher than for the conventional order group ($Z = -2.368$, $p = 0.018$, $n = 19$), with a trend in

Table 4. Treatment order effect: *post hoc* analyses within group differences in bilateral [18F]FDOPA *K*

Group	[18F]FDOPA *K*	No drug	SD	*N*	MPH	SD	*N*	Change (Δ)	% Change	*Z*	*p*
CTR-MPH	CN	0.0204	0.0031	14	0.0218	0.0038	14	0.0014	7%	−1.083	0.279
	PUT	0.0186	0.0035	14	0.0195	0.0035	14	0.0009	5%	−1.642	0.101
MPH-CTR	CN	0.0273	0.0063	6	0.0224	0.0030	6	−0.0049	−18%	−1.782	0.075
	PUT	0.0230	0.0056	6	0.0200	0.0018	6	−0.0030	−13%	−1.992	0.046*

CTR, No drug condition; CN, caudate nucleus; PUT, putamen; MPH, methylphenidate condition.
*$p < 0.05$.

Table 5. Correlations between neuropsychological parameters and [18F]FDOPA *K* in bilateral CN and PUT

	CN						PUT					
	Bilateral	*p*	Right	*p*	Left	*p*	Bilateral	*p*	Right	*p*	Left	*p*
TMT-A	0.093	NS	0.082	NS	0.258	NS	0.14	NS	0.21	NS	−0.027	NS
TMT-B	−0.495	0.086	−0.599	0.031*	−0.154	NS	−0.545	0.067	−0.497	NS	−0.604	0.029*
d2 CP	0.492	0.087	0.583	0.036*	0.294	NS	0.75	0.005**	0.701	0.011	0.707	0.007**
Decision bias β	0.214	NS	0.192	NS	0.154	NS	−0.056	NS	0.007	NS	−0.016	NS
Discriminability d′	−0.027	NS	−0.044	NS	0.099	NS	−0.259	NS	−0.273	NS	−0.236	NS

CN, Caudate nucleus; PUT, putamen; NS, not significant.
*$p < 0.05$; **$p < 0.01$.

bilateral caudate nucleus ($Z = -1.842$, $p=0.072$, $n = 19$, Wilcoxon test). Insofar as this may indicate a carryover effect 2 weeks after MPH on [18F]FDOPA kinetics, we analyzed *post hoc* differences for both groups (Table 4). The results indicate that the increase was *K* only significant in the reversed-order group.

Relationships between cognitive performance and baseline *K*

Because of the order of treatment effect, correlations between *K* and neuropsychological measures were conducted only within the conventional order group ($n = 14$). We found that baseline magnitude of *K* in left putamen correlated negatively with baseline TMT-B: the lower *K*, the better the performance (Fig. 1*b*) (TMT-B is measured in seconds, such that briefer duration of the test indicated better working memory). Furthermore, there was a negative correlation between CP (d2 test) scores and *K* in left and right putamen (Table 5; Fig. 1*c*).

Correlations with change in k_{loss} and change in neuropsychological measurements

There was a strong correlation between Δ-k_{loss} in bilateral putamen and the improvement in CP under MPH ($r = 0.599$, $p = 0.040$, $n = 12$, Spearman; Fig. 1*d*). Furthermore, Δ-k_{loss} in right putamen correlated negatively with the change in decision bias (Δ-β) within the conventional order group ($r = -0.630$, $p = 0.028$, $n = 12$, Spearman).

Discussion

Using [18F]FDOPA PET in conjunction with the IOM approach for estimation of the dopamine turnover, the present study was designed to quantify direct effects of acute MPH on nigrostriatal dopamine metabolism in healthy females and to link these pharmacodynamic effects to individual cognitive performance changes. We found the expected decrease of k_{loss} in the MPH condition. Most likely, k_{loss} is decreased because of particular action of MPH as a reuptake inhibitor, which decreases the reentry of released dopamine into the intracellular substrate pool for monoamine oxidase. Thus, under MPH, the oxidative deamination of released dopamine to the diffusible metabolite DOPAC by monoamine oxidase is largely disabled (Nielsen et al., 1983; Zetterström et al., 1988). Furthermore, autoreceptor-mediated feedback reduces the formation of diffusible dopamine metabolites. Nonetheless, the magnitude of *K* was unaffected by acute MPH treatment, such that the observed changes of volume of distribution (V_d) are mostly driven by k_{loss}. Baseline parameters for attention performance (CP [d2 test] and TMT-B) correlated with baseline [18F]FDOPA *K* in putamen; similar results for other cognitive parameters are found in previous studies (Vernaleken et al., 2007, 2008). We also detected significant correlations between [18F]FDOPA Δ-k_{loss} and change of cognitive performance parameters evoked by MPH: in particular, the individual change of the decision bias ($\Delta\beta$) and the extent of k_{loss} reduction correlated positively. Furthermore, ΔCP correlated strongly with Δk_{loss}, indicating more pronounced attentional improvement in subjects with the greatest pharmacodynamic effect of MPH. Interestingly, our randomized study design revealed a strong PET scan-ordering effect, such that there was an apparent increase in drug-free [18F]FDOPA *K* (the dopamine synthesis capacity) persisting at 2 weeks after a single, moderate MPH dose.

It is well known that MPH inhibits the plasma membrane catecholamine transporters, causing an increase of intrasynaptic dopamine and noradrenaline concentrations. Volkow et al. (1998, 1999) described DAT occupancy of up to 74% in human subjects treated with MPH. Microdialysis studies have shown doubling of the interstitial dopamine concentration in rat striatum after oral MPH administration (Gerasimov et al., 2000; Berridge et al., 2006). Previous $D_{2/3}$-receptor ligand PET studies have shown availability decreases in healthy subjects ranging from 6% to 27% with MPH challenge (Volkow et al., 1994; Udo de Haes et al., 2005; Del Campo et al., 2011). The construct validity of the competition paradigm, however, has some caveats because results depend on which $D_{2/3}$ ligand is used (Gjedde and Wong, 1987; Morris and Yoder, 2007) and an imperfect relation between interstitial dopamine changes and the time course of alterations in receptor availability seen by PET exists (Laruelle et al., 1997; Houston et al., 2004). This discrepancy may be related to receptor internalization and affinity states induced by dopamine agonists. Studies showed internalization effects for amphetamine, dopamine, and dopamine agonists (Bartlett et al., 2005; Skinbjerg et al., 2010). Given these vagaries, we proposed that the [18F]FDOPA IOM should provide a more interpretable assay of the pharmacodynamic effects of a psychostimulant challenge. Indeed, the percentage change of k_{loss} exceeded that of the $D_{2/3}$-receptor availability seen in many MPH challenge studies.

The parameter k_{loss} reflects the composite of partitioning of [18F]FDOPA between cytosolic and vesicular compartments, release and reuptake, subsequent exposure to monoamine oxidase, and diffusion of deaminated metabolites from brain (Deep et al., 1997). Despite this complexity, we have shown that the k_{loss} magnitude depicts the steady-state fractional rate constant for dopamine turnover (Deep et al., 1997). As such, our observations of a positive association between decrease of k_{loss} and MPH-related modulations of cognitive performance link the pharmacodynamic effect of MPH on dopamine metabolism; the more pronounced the decrease in k_{loss}, the less improvement in concentration performance was observed. Analogously, a previous [18F]FDOPA IOM study showed that individual changes in k_{loss} following haloperidol challenge likewise correlate with cognitive changes (Vernaleken et al., 2008). In the present study, MPH did not evoke cognitive improvement in every case; we suppose that many in our cohort were high achievers with normal IQ, and already in a state of optimal dopamine balance, which could not be improved by MPH challenge. Using the SDT analyses, we detected another important association consistent with procognitive and attention effects of MPH in ADHD patients; participants who manifested the strongest impact of MPH in reducing k_{loss} also showed the strongest shift to a more conservative decision bias. The decision bias provides a measure of how liberal or conservative the decisions were, regardless of overall performance. These findings might be relevant for several psychiatric disorders that include cognitive disabilities (van Beilen et al., 2008; Jokinen et al., 2013; Kumakura et al., 2013).

The widespread clinical use of psychostimulants in ADHD has raised concerns with respect to long-term treatment effects. Behavioral sensitization to amphetamine is well known in rodent studies (Robinson et al., 1982), and previous MPH treatment evokes persistent changes in interstitial dopamine (Calipari et al., 2013, 2014) and NMDA receptors in rat brain (Urban et al., 2013). A human PET study revealed potentiation of reductions in amphetamine-evoked $D_{2/3}$-receptor availability some months after the previous dose of amphetamine, indicating persistent sensitization (Boileau et al., 2006). Based on these results, our study design included a subgroup with a reversed order of the drug-free and pharmacological challenge [18F]FDOPA PET scans. We did see an order effect on the magnitude of K; MPH treatment 2 weeks before the scan significantly (~18%) increased striatal dopamine synthesis capacity. This presynaptic change might have arisen through feedback regulation of dopamine autoreceptors, which could likewise be a factor in the amphetamine sensitization reported by Boileau et al. (2006). Verification of the presynaptic potentiation would require prospective [18F]FDOPA studies with repeated psychostimulant challenge. Ernst et al. (1999) found that ADHD patients had increased [18F]FDOPA uptake in midbrain compared with healthy subjects, despite being drug free for several weeks. Present findings suggest that this, too, may have reflected a carryover effect rather than an ADHD trait per se. Our main endpoint in the present study, k_{loss}, obviously is a more volatile parameter, being related to present state of dopamine turnover. Consistent with this conception, we saw no evidence for a carryover in the magnitude of k_{loss}. If further studies replicate our finding of persistently increased dopamine synthesis capacity following MPH, it will become an issue whether this effect contributes to the beneficial clinical effects in ADHD, which are usually attributed to the acute increase of dopamine levels.

Some limitations of the present study should be noted. In general, the complex nature of [18F]FDOPA metabolism in brain (Cumming et al., 1987) hampers the interpretation of our findings. The IOM, which accommodates the reversibility of [18F]FDOPA trapping, is necessarily a simplification of the biological complexity of dopamine metabolism. As noted above, our main endpoint k_{loss} is explicable as an index of dopamine turnover, traced by the elimination of the [18F]FDOPA pool formed in striatum, such that a reduction of k_{loss} reflects a decline in turnover. Our previous results confirm the (patho)physiological relevance of disturbed k_{loss} in psychiatric and neurological disorders (Kumakura et al., 2007). In contrast, the [18F]FDOPA parameter K represents the capacity to use (exogenous) [18F]FDOPA in brain, which is hardly subject to regulation upon acute MPH challenge. We previously reported changes in [18F]FDOPA K upon treatment for 3 d with a $D_{2/3}$-anatgonist (Vernaleken et al., 2008), whereas short-term antipsychotic treatment had no effect on corresponding m-tyrosine PET findings (Mamo et al., 2004; Vernaleken et al., 2006). Our design deliberately entailed a test of order effect on [18F]FDOPA kinetics, which revealed k_{loss} to be robust to this factor, whereas K was increased by previous MPH exposure. Our subjects did not otherwise report any previous use of psychostimulants, so we feel confident in our observation of a main effect of MPH challenge on k_{loss}. In clinical [18F]FDOPA studies of ADHD patients, drug treatment history is very likely to be relevant, given that K was strongly affected by previous one-time MPH use. Nevertheless, the unequal group sizes call for caution in the interpretation of this finding. The group with the reversed treatment order ($n = 6$) was smaller than the group with standard scanning order ($n = 14$). This was, by design, to provide sufficient power for testing the main hypotheses concerning correlations between PET and cognitive changes. Also notably, the test–retest variability is 10% for conventional reference tissue [18F]FDOPA PET (Egerton et al., 2010); the corresponding covariance remains to be established for the IOM method. Given that a possibly higher variance in the outcome parameters of the IOM might exist, which would impair the test–retest reliability, from a statistical point of view, this would not relativize the fact that, based on the present observations, the probability to reject a possible true null hypothesis is <5% (risk of false positive as depicted by $\alpha \leq 0.05$). Lower test–retest reliability, however, depends on higher necessary effects to contrast against the higher level of noise in the data.

Notes

Supplemental material for this article is available at http://www.ukaachen.de/fileadmin/files/klinik-psychiatrie/Download_Personen/The_reversible_Inlet-Outlet_Model.pdf. Detailed description of the reversible Inlet/Outlet Model. Giving a more complete overview and a better understanding of the kinetic model which is used in this manuscript. This material has not been peer reviewed.

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